Assessing the clinical relevance of Fenollaria massiliensis in human infections, using MALDI-TOF MS

Boiten, K. E.; Jean-Pierre, H.; Veloo, A. C. M.

Published in:
Anaerobe

DOI:
10.1016/j.anaerobe.2018.03.008

Publication date:
2018

Anaerobe 54 (2018) 240–245

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Clinical microbiology

Assessing the clinical relevance of *Fenollaria massiliensis* in human infections, using MALDI-TOF MS

K.E. Boiten [a, *], H. Jean-Pierre [b], A.C.M. Veloo [a]

[a] University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, The Netherlands
[b] Centre Hospitalier Universitaire de Montpellier, Hôspital Arnaud de Villeneuve, Laboratoire de Bactériologie, 371 Avenue du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 15 January 2018
Received in revised form
15 March 2018
Accepted 16 March 2018
Available online 17 March 2018

Handling Editor: Elisabeth Nagy

Keywords:
Fenollaria massiliensis
MALDI-TOF MS
ENRIA
Less common species
Clinical relevance

ABSTRACT

Within the European Network for the Rapid Identification of Anaerobes (ENRIA) project eight clinical isolates of *Fenollaria massiliensis* were encountered. In this study a more extensive description of this species is given and the MALDI-TOF MS database is optimized for its identification.

F. massiliensis is an anaerobic Gram positive rod with the tendency to decolorize quickly. It is mostly encountered in clinical samples from the groin region.

Less common and non-valid species are not represented in the MALDI-TOF MS database. Therefore, *F. massiliensis* can only be identified by laboratories performing 16S rDNA gene sequencing. The addition of less common and non-valid species to the database will give insight in their clinical relevance.

1. Introduction

Due to the introduction of Matrix Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) in diagnostic laboratories, species which previously remained unidentified can now be identified easily [14,16]. Until now, it was only possible to identify relatively unknown species by 16S rDNA gene sequencing, which is expensive and time consuming in comparison to MALDI-TOF MS [10].

However, identification can only be performed if the database accompanying MALDI-TOF MS systems contain a sufficient number of high quality Main Spectral Profiles (MSPs) representing species encountered in human clinical specimens [10].

The goal of the European Network for the Rapid Identification of Anaerobes (ENRIA) project is to optimize the MALDI-TOF MS database for the identification of anaerobes. Within this project anaerobic clinical isolates were encountered which could previously not be identified using MALDI-TOF MS, for example *Fenollaria massiliensis*.

Due to the optimization of the MALDI-TOF MS database we encountered eight clinical isolates of *F. massiliensis*. The initial description of this species was based on one clinical isolate, rendering its clinical relevance unknown [7].

In this study a more extensive description of the biochemical features and an insight in the clinical relevance of *F. massiliensis* is given. Furthermore, the MALDI-TOF MS database is optimized for the identification of this species.

2. Material and methods

2.1. Bacterial strains

Eight strains of *F. massiliensis* were isolated from clinical samples, six strains at the Centre Hospitalier Universitaire de Montpellier, France and two strains at the University Medical Center Groningen, The Netherlands. The strains originated from bone tissue, a perianal abscess, a vulva abscess, a cutaneous flap infection, a cutaneous fistula, a post-operative scar and two strains from sperm samples. Furthermore, the type strain *F. massiliensis* CSUR P127^T was included in all tests.

* Corresponding author. University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
E-mail address: k.e.boiten@umcg.nl (K.E. Boiten).

https://doi.org/10.1016/j.anaerobe.2018.03.008

All strains were cultured on Brucella Blood Agar ((BBA) Mediaproducts BV, Groningen, The Netherlands) supplemented with hemin (5 mg/L) and vitamin K (10 mg/L), and incubated in anaerobic conditions (80% N_2, 10% H_2, and 10% CO_2) at 35 °C for 48 h.

2.2. Biochemical features

The special potency discs; vancomycin (5 μg), kanamycin (1000 μg), colistin (10 μg) and bile, were used for preliminary identification of anaerobic bacteria, according to the wadsworth manual [4].

Biochemical features were assessed using the rapid ID 32A (bioMerieux S.A., Marcy l'Etoile, France), according to the recommendations of the manufacturer.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using Etests® (bioMerieux S.A., Marcy l'Etoile, France). Susceptibility to amoxicillin, amoxicillin-clavulanic acid, clindamycin, metronidazole, meropenem and tetracycline was assessed. All Etests® were incubated for 48 h in an anaerobic environment on BBA agar. Minimal Inhibitory Concentrations (MICs) were interpreted according to the breakpoints provided by EUCAST (http://www.eucast.org/), except for tetracycline. Since EUCAST gives no breakpoints for tetracycline, the CLSI guidelines were used. Beta-lactamase activity was tested using BBL cefinase discs (Becton, Dickinson and Company, US).

2.4. DNA isolation, 16S rDNA gene sequencing and phylogenetic analysis

DNA of FR0557 and UMCG-9195 was extracted as described by Boom et al. [1]. 16S rDNA gene sequencing of strains was performed at the UMCG using universal primers. Of strain FR0557 the whole 16S rDNA gene was sequenced [9] and of strain UMCG-9195 only the first 500bp [2]. The identity of the strains was confirmed using BLASTn, hereby comparing the obtained 16S rDNA sequences with reference sequences available in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The threshold value for a reliable species identification was set at >98,7% [12].

Using MEGA 7 [5], a phylogenetic tree was constructed. All sequence fragments were aligned and a filter was set to ensure equal length. Branching was determined using the neighbor-

Table 1
Clinical information of the *F. massiliensis* strains.

Strain	Gender (age)	Origin	Other pathogens	
			Aerobic	Anaerobic
UMCG-9195	Male (48)	bone (tibia) tissue from a patient with chronic osteomyelitis	*Corynebacterium aurimucosum* *Dermabacter hominis* *Staphylococcus simulans* *Streptococcus mitis* group	*F. massiliensis* *Actinomyces turicensis* *Actinotignum schaalii* *Anaerococcus lactolyticus* *Peptoniphilus harei*
UMCG-6509	Female (33)	Pus perianal abscess	Aerobic Gram positive mixture *Cronobacter sakazakii*	*F. massiliensis* Anaerobic gram positive rod *Bilophila wadsworthia* *Campylobacter ureolyticus* *Peptostreptococcus anaerobius* *Veillonella parvula*
FR5551	Female (33)	vulva abscess	*Gardnerella vaginalis* *Staphylococcus caprae*	*F. massiliensis* *Peptoniphilus* spp. *Porphyromonas asaccharolyticus* *Cutibacterium avidum*
FR5612	Male (35)	sperm	*Streptococcus oralis*	*F. massiliensis* *A. lactolyticus*
FR6112	Male (35)	sperm		*F. massiliensis* *Murdochiella asaccharolytica* *Peptoniphilus gorbachii* *Porphyromonas bennonis* *Prevotella* spp. *Prevotella corporis*
FR6108	Male (50)	Infection on a cutaneous flap (ischium level)		*F. massiliensis* *Finegoldia magna* *P. lacrimalis*
FR6144	Male (66)	Suppuration of a cutaneous fistula after curing inguinal hernia	*Staphylococcus epidermidis*	*F. massiliensis* *Anaerococcus vaginalis* *Eubacterium* spp. *Peptoniphilus* spp. *Porphyromonas somerae* *P. corporis* *C. avidum*
FR0557	unknown	post-operative scar		*F. massiliensis* Gram positive anaerobic cocci

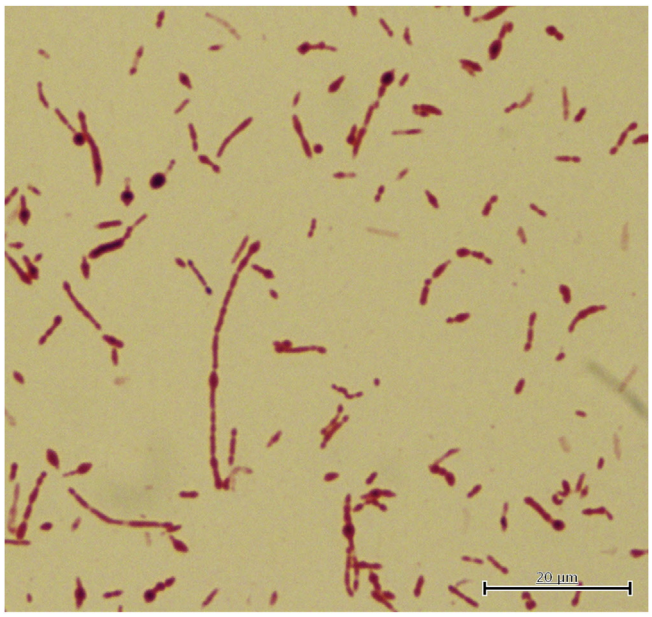

Fig. 1. Gram stain of *F. massiliensis* strain UMCG-9195.

Table 2
Biochemical features of *F. massiliensis* determined by rapid ID 32A.

	F. massiliensis Pagnier et al. [6]	CSUR P127^T	ENRIA isolates
URE	−	−	−
ADH	−	+	+
αGAL	−	−	−
βGAL	−	−	−
βGP	−	−	−
αGLU	−	−	−
βGLU	−	−	−
αARA	−	−	−
βGUR	−	−	−
βNAG	−	+	v
MNE	−	−	−
RAF	−	−	−
GDC	−	−	−
αFUC	−	−	−
NIT	−	−	−
IND	+	+	v
PAL	−	−	−
ArgA	+	+	+
ProA	−	−	−
LGA	−	+	+
PheA	−	+	+
LeuA	+	+	+
PyrA	+	+	+
TyrA	+	+	+
AlaA	−	+	+
GlyA	+	+	+
HisA	+	+	+
GGA	−	+	+
SerA	+	+	+

v: variable, URE: urease, ADH: arginine dihydrolase, αGAL: α-galactosidase, βGAL: β-galactosidase, βGP: β-galactosidase phosphate, αGLU: α-glucosidase, βGLU: β-glucosidase, αARA: α-arabinosidase, βGUR: β-glucuronidase, βNAG: N-acetyl-β-glucosaminidase, MNE: mannose fermentation, RAF: raffinose fermentation, GDC: glutamic acid decarboxylase, αFUC: α-fucosidase, NIT: nitrate reduction, IND: indole production, PAL: alkaline phosphatase, ArgA: arganine arylamidase, ProA: Proline arylamidase, LGA: leucyl glycine arylamidase, PheA: Phenylalanine arylamidase, LeuA: leucine arylamidase, PyrA: pyroglutamic acid arylamidase, TyrA: tyrosine arylamidase, AlaA: alanine arylamidase, GlyA: glycine arylamidase, HisA: histidine arylamidase, GGA: glutamyl glutamic acid arylamidase, SerA: serine arylamidase.

joining method with a bootstrap test of 500. *F. massiliensis* strain FR0557, the type strains of *F. massiliensis* 9401234^T (NR_133038) and *F. timonensis* GD5^T (LN 881613), and anaerobic species belonging to the same family were included.

2.5. MALDI-TOF MS

2.5.1. MSP creation

A full extraction of the strains was performed as described previously by Veloo et al. [15]. Briefly, an ethanol suspension was made by dissolving a 1 µl loop full of bacteria in 300 µl sterile distilled water. After obtaining a homogeneous suspension 900 µl of pure ethanol was added. The suspension was centrifuged for 2 min at 13.000 g and the supernatant was removed. This step was repeated and the supernatant was carefully removed by pipetting. The pellet was dissolved in 30 µl of 70% formic acid. An equal amount of acetonitrile was added and the suspension was centrifuged for 2 min at 13.000 g. 1 µl of the supernatant was spotted twelve times on a steel 96 wells target and dried at ambient temperature. Immediately after drying 1 µl of α-cyano-4-hydroxycinnamic acid (HCCA) matrix was added and left to dry at ambient temperature.

Each spot was measured 3 times using a microflex LT/SH MALDI-TOF MS system (Bruker daltonik GmbH, Bremen, Germany), resulting in 36 spectra. Prior to the measurement the MALDI-TOF MS system was calibrated using the Bacterial test standard (BTS, Bruker daltonik GmbH, Bremen, Germany).

Using FlexAnalysis 3.4 a smoothing and baseline subtraction was performed on the spectra in accordance with FlexAnalysis method MBT_standard. The spectra were manually evaluated. All flatliners and remarkably different spectra were discarded from the dataset. The remaining spectra were checked for peak shifts, which should be less than 500 ppm. All spectra that exceeded this value were discarded from the data set as well. A MSP was calculated of at least 20 spectra using MBT Compass Explorer 4.1.

A dendrogram was created of the in house made *F. massiliensis* MSPs and MSPs of anaerobic species, which were also used to calculate the phylogenetic tree, using MBT Compass Explorer. Furthermore, two MSPs for *F. timonensis*, available at the website http://www.mediterranee-infection.com/article.php?larub=280&titre=urms-database from Durand et al. [3], were downloaded and added to the dendrogram.

2.5.2. Strain identification

The MSPs of strains FR0557 and UMCG-9195 were used to identify the six other *F. massiliensis* strains. The MALDI-TOF MS measurements were performed as previously described by Veloo et al. [13]. After 48 h of subculturing on BBA, each strain was spotted in duplicate on a stainless steel target plate. 1 µl of HCCA matrix was

Table 3
MIC values for different antibiotics.

Strain	MIC (mg/L)					
	AC	XL	CM	MZ	MP	TE
CSUR P127^T	<0.016	<0.016	1.0	0.094	<0.002	16
UMCG-9195	<0.016	<0.016	1.0	<0.016	<0.002	0.125
UMCG-6509	0.023	0.016	0.38	0.094	<0.002	0.5
FR5551	0.016	0.016	0.5	0.047	<0.002	0.25
FR5612	<0.016	<0.016	0.032	0.016	<0002	0.032
FR6112	<0.016	<0.016	16	0.047	<0.002	8.0
FR6108	0.032	0.032	0.5	0.125	0.004	0.75
FR6144	0.016	0.023	>256	0.19	<0.002	0.25
FR0557	<0.016	0.016	1.5	0.19	0.004	0.38

AC: Amoxicillin, XL: Amoxicillin clavulanic acid, CM: Clindamycin, MZ: Metronidazole, MP: Meropenem, TE: Tetracycline.

added to the spot and left to dry at ambient temperature.

The MALDI-TOF MS results were interpreted as recommended by the manufacturer (Bruker daltonik GmbH, Bremen, Germany). Log scores ≥2.0 were considered as an identification with high confidence, log scores of ≥1.7–2.0 were considered as an identification with low confidence. Log scores <1.7 were considered as no reliable identification.

3. Results

3.1. Bacterial strains

All strains were isolated from mixed infections with either anaerobic bacteria or both aerobic and anaerobic bacteria (Table 1). In three clinical samples solely anaerobic isolates were encountered. Furthermore, from each clinical sample Gram positive anaerobic cocci (GPAC) were isolated and two contained besides *F. massiliensis* only GPAC.

3.2. Biochemical features

All *F. massiliensis* strains showed good growth after 48 h of incubation on BBA. Colonies were small, circular, bright greyish, non-hemolytic with a diameter of 0.5–1.5 mm. All strains were catalase negative. The Gram stain showed pleomorphic Gram positive rods with round bodies on filaments (Fig. 1). The bacterial cells show a tendency to decolorize easily. All strains were sensitive to vancomycin, kanamycin and bile but resistant to colistin using the special potency discs.

Using rapid ID 32A all strains showed positive reactions for arginine dihydrolase, arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glytamyl glutamic acid arylamidase and serine arylamidase. Negative reactions were observed for urease, α-galactosidase, β-galactosidase, β-galactosidase phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, mannose, raffinose, glutamic acid decarboxylase, α-fucosidase, nitrate, alkaline phosphatase and proline arylamidase. Variable results were observed for N-acetyl-β-glucosaminidase and indole production. The type strain CSUR P127^T showed similar results in the rapid ID 32A (Table 2).

3.3. Antimicrobial susceptibility

All strains produced no beta-lactamase and were susceptible for amoxicillin, amoxicillin/clavulanic acid, metronidazole and meropenem. Two of the eight clinical strains were resistant to clindamycin, MICs of 16 mg/L and >256 mg/L, and one strain was intermediate to tetracycline, MIC of 8 mg/L. The type strain CSUR P127^T was susceptible to all tested antibiotics, except for tetracycline, for which a MIC of 16 mg/L was observed (Table 3).

3.4. MALDI-TOF MS

No reliable identification was obtained using the MALDI-TOF MS with the Bruker version 6 (6903 MSPs) database. After the addition of in house made *F. massiliensis* MSPs of strains UMCG-9195 and FR0557 to the database, all strains, including the type strain, could be identified with a high confidence log score. Comparing the obtained spectra of all *F. massiliensis* strains to the MSP of *F. timonensis*, yielded a log score <1.7.

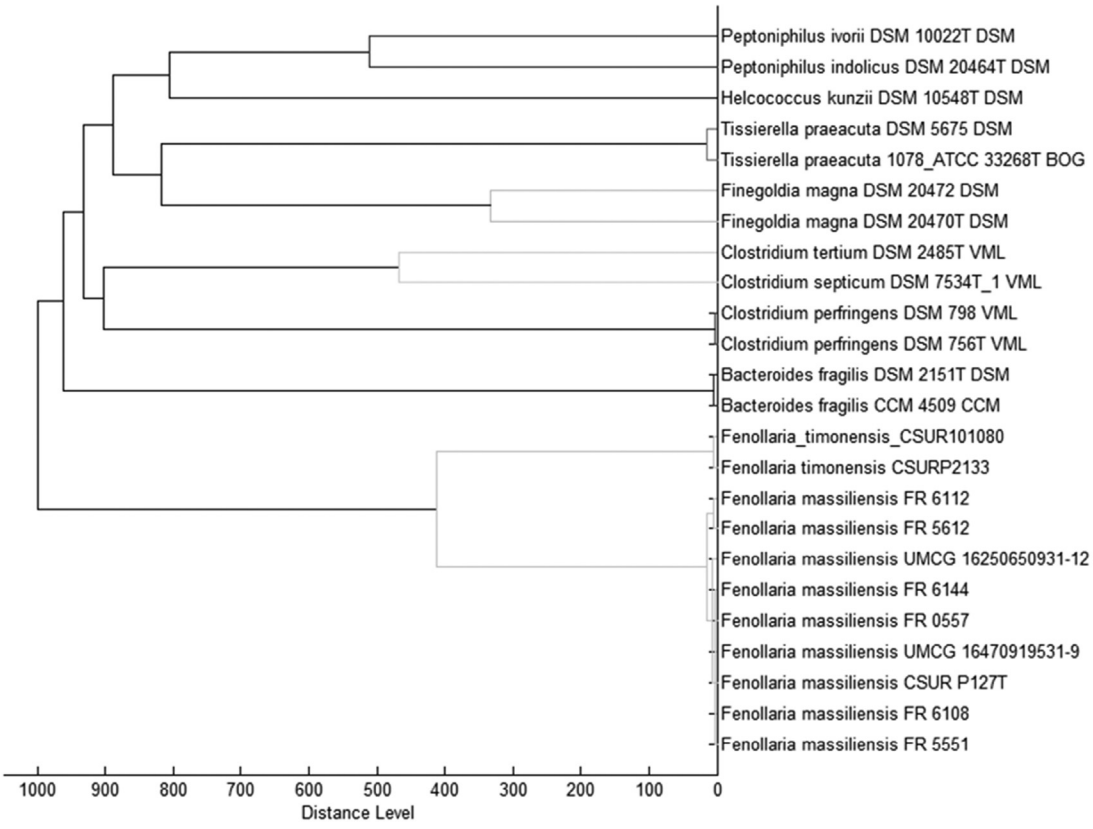

Fig. 2. Dendrogram of all *F. massiliensis* strains and related anaerobic species.

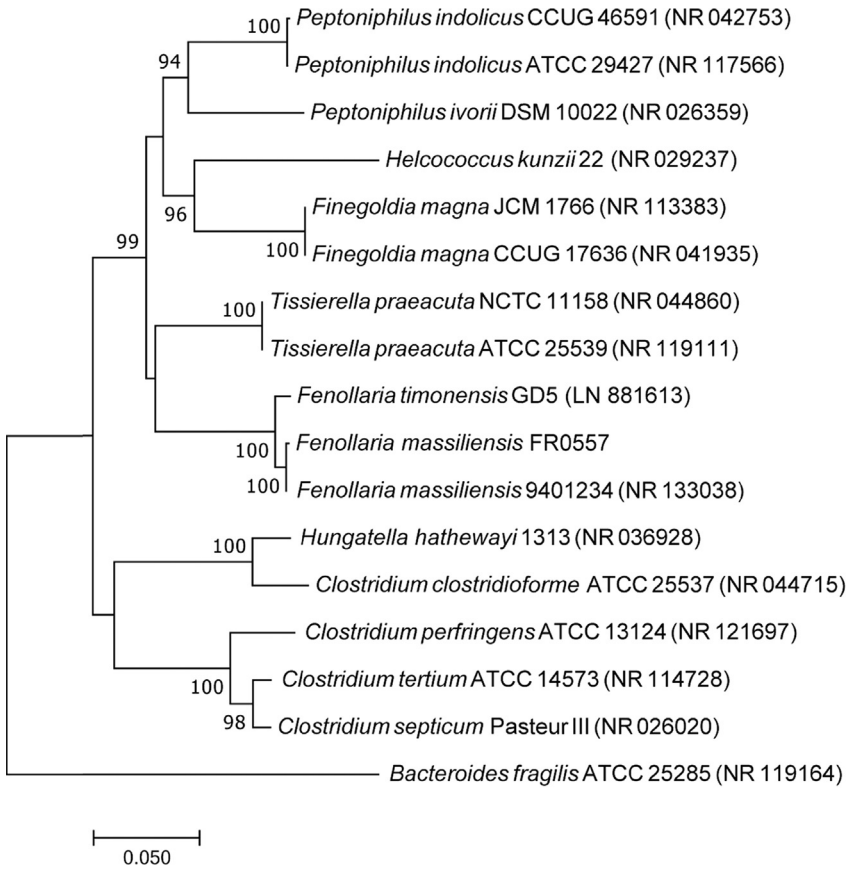

Fig. 3. Phylogenetic tree showing the relationship of *F. massiliensis* with related anaerobic species. Only bootstrap values of >90% are shown.

The obtained dendrogram is shown in Fig. 2. The in house created MSPs of *F. massiliensis* cluster closely together and show a close relation to *F. timonensis*. A clear difference was seen with the *Fenollaria* strains and other genera.

3.5. 16S rDNA sequencing

Strains FR0557 and UMCG-9195 each had a sequence similarity of 99% with *F. massiliensis*.

The obtained phylogenetic tree is shown in Fig. 3. Strain FR0557 clusters closely together with the type strain of *F. massiliensis* and shows a close relationship with the type strain of *F. timonensis*.

4. Discussion

In this study, the biochemical characteristics and clinical relevance of *F. massiliensis* are described.

F. massiliensis is a relatively unknown anaerobic bacterium, originating from an osteoarticular sample from a patient in France [7] and was classified as being a Gram negative rod. The genus *Fenollaria* consists out of two species: *F. massiliensis* and *F. timonensis* [3]. Due to the introduction of molecular techniques more and more new species are described. It is of the outmost importance that new species are validated by the International Journal of Systemic and Evolutionary Microbiology (IJSEM) and accepted by the International Committee on Systemics of Prokaryotes (ICSP, http://www.the-icsp.org/). *F. massiliensis* was recently published in IJSEM as a valid species [6]. However, *F. timonensis* is still a non-valid species. Non-valid species are not represented in the MALDI-TOF MS database and are therefore not identified by laboratories not performing 16S rDNA gene sequencing. We showed that, when using an improved database, *F. massiliensis* can easily be identified using MALDI-TOF MS. The addition of less common and non-valid species facilitates the assessment of the clinical relevance of such species.

We noticed that the first cultures of *F. massiliensis* yielded Gram positive stained cells which after several sub cultures stained Gram negative.

Analysis of the genome (NZ_CALI00000000) yielded five genes for a Gram positive cell wall (all belonging to the family of glycosyltransferase) and only one gene for a Gram negative cell wall (data not shown). Glycosyltransferases play an important role in the biosynthesis of wall teichoid acids, which are an important part of the peptidoglycan layer of Gram positive bacteria [11]. The wadsworth manual [4] mentions that Gram positive organisms are most likely to be sensitive to the special potency disc vancomycin, whereas Gram negative organisms are most likely to be resistant. Since several Gram positive anaerobic bacteria have the tendency to decolorize easily in a Gram stain, we suspect that this is also the case with *F. massiliensis* and was therefore mistaken to be a Gram negative species.

The obtained biochemical features of the type strain of *F. massiliensis* were similar as the ones obtained for the clinical isolates. However, we observed positive reactions for arginine dihydrolase, N-acetyl-β-glucosaminidase, leucyl glycine arylamidase, phenylalanine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase, which is in contrast with the biochemical features described by Pagnier et al. [7]. The type strain of *F. massiliensis* was the only strain resistant to tetracycline.

Six *F. massiliensis* strains were isolated from clinical samples

originating from the groin region. It is interesting to notice that Sabat et al. [8] recently encountered *F. massiliensis* in urine samples using direct targeted next-generation sequencing. In this study, four out of sixty urine samples were shown to contain DNA of *F. massiliensis*, ranging from 15.6% till 0.4% of the total bacterial DNA. Taking also our findings into the account, this might indicate that the groin region is the commensal habitat of *F. massiliensis*.

In summary, *F. massiliensis* is an anaerobic Gram positive rod present in the groin region. This study shows the importance of adding MSPs of less common and non-valid species to the MALDI-TOF MS database.

References

[1] R. Boom, M.M. Salimans, C.L. Jansen, P.M. Werheim-van Dillen, J. van der Noordaa, Rapid and simple method for purification of nucleic acids, J. Clin. Microbiol. 28 (3) (1990) 495–503.

[2] L. Chen, Y. Cai, G. Zhou, X. Shi, J. Su, G. Chen, K. Lin, Rapid sanger sequencing of the 16S rRNA gene for identification of some common pathogens, PLoS One 9 (2) (2014), e88886, https://doi.org/10.1371/journal.pone.0088886.

[3] G.A. Durand, F. Cadoret, J.C. Lagier, P.E. Fournier, D. Raoult, Description of '*Gorbachella massiliensis*' gen. nov., sp. nov., '*Fenollaria timonensis*' sp. nov., '*Intestinimonas timonensis*' sp. nov. and '*Collinsella ihua*' sp. nov. isolated from healthy fresh stools with culturomics, New Microbe and New Infect 16 (2017) 60–62, https://doi.org/10.1016/j.nmni.2017.01.005.

[4] H.R. Jousimies-Somer, P. Summanen, D.M. Citron, E.J. Baron, H.M. Wexler, S.M. Finegoldii, Wadsworth-KTL Anaerobic Bacteriology Manual, sixth ed., Star publishing, Belmont, Canada, 2002, pp. 55–74. Chapter 4, pg.

[5] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version7.0 for bigger datasets, Mol. Biol. Evol. 33 (7) (2016) 1870–1874, https://doi.org/10.1093/molbev/msw054.

[6] A. Oren, G.M. Garrity, List of new names and new combinations prevously effectively, but not validly, published. Validation list no. 180, Int. J. Syst. Evol. Microbiol. 68 (2018) 693–694.

[7] I. Pagnier, O. Croce, C. Robert, D. Raoult, B. La Scola, Non-contiguous finished genome sequence and description of *Fenollaria massiliensis* gen. nov., sp. nov., a new genus of anaerobic bacterium, Stand Genomic Sci 9 (2014) 704–717, https://doi.org/10.4056/sigs.3957647.

[8] A.J. Sabat, E. van Zanten, V. Akkerboom, G.J. Wisselink, K. van Slochteren, R.F. de Boer, R. Hendrix, A. Friedrich, J. Rossen, A. Kooistra-Smid, Targeted next-generation sequencing of the 16-23S rRNA region for culture-independent bacterial identification – increased discrimination of closely related species, Sci. Rep. 7 (1) (2017) 3434, https://doi.org/10.1038/s41598-017-03458-6.

[9] T. Schuurman, R.F. de Boer, A.M. Kooistra-Smid, A.A. van Zwet, Prospective study of use of PCR amplification and sequencing of 16S ribosomal DNA from cerebrospinal fluid for diagnosis of bacterial meningitis in a clinical setting, J. Clin. Microbiol. 42 (2) (2004) 734–740, https://doi.org/10.1128/JCM.42.2.734-740.2004.

[10] P. Seng, M. Drancourt, F. Gouriet, B. La Scola, P.E. Fournier, J.M. Rolain, D. Raoult, Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption time-of-flight mass spectrometry, Clin. Infect. Dis. 49 (4) (2009) 543–551, https://doi.org/10.1086/600885.

[11] J.G. Swoboda, J. Campbell, T.C. Meredith, S. Walker, Wall Teichoic Acid function, biosynthesis, and inhibition, Chembiochem 11 (1) (2010) 35–45, https://doi.org/10.1002/cbic.200900557.

[12] E. Stackebrandt, J. Ebers, Taxonomic parameters revisited: tarnished gold standards, Microbiol. Today 33 (4) (2006) 152–155.

[13] A.C.M. Veloo, P.E. Elgersma, A.W. Friedrich, E. Nagy, A.J. van Winkelhoff, The influence of incubation time, sample preparation and exposure to oxygen on the quality of the MALDI-TOF MS spectrum of anaerobic bacteria, Clin. Microbiol. Infect. 20 (12) (2014) 1091–1097, https://doi.org/10.1111/1469-0691.12644.

[14] A.C. Veloo, M. Erhard, M. Welker, G.W. Welling, J.E. Degener, Identification of gram-positive anaerobic cocci by MALDI-TOF mass spectrometry, Syst. Appl. Microbiol. 34 (1) (2011) 58–62, https://doi.org/10.1016/j.syapm.2010.11.005.

[15] A.C.M. Veloo, E.D. de Vries, H. Jean-Pierre, U.S. Justesen, T. Morris, E. Urban, I. Wybo, A.J. van Winkelhoff, The optimization and validation of the Biotyper MALDI-TOF MS database for the identification of gram-positive anaerobic cocci, Clin. Microbiol. Infect. 22 (2016) 793–798, https://doi.org/10.1016/j.cmi.2016.06.016.

[16] I. Wybo, O. Soetens, A. De Bel, F. Echalidi, E. Vancutsem, K. Vandoorslaer, D. Piérard, Species identification of clinical *prevotella* isolates by matrix assisted laser desorption ionization-time of Flight mass spectrometry, J. Clin. Microbiol. 50 (4) (2012) 1415–1418, https://doi.org/10.1128/JCM.06326-11.